

ANALYSIS OF THE ORGANIC ACIDS OF ORANGE JUICE¹

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(WITH FOUR FIGURES)

The total acidity of orange juice is ordinarily determined by titrating a known volume of juice with a standard solution of NaOH, with phenolphthalein as indicator, the result being expressed as citric acid. This value includes all the substances of an acidic nature in the juice that react with NaOH. It so happens, however, that in most fruits the chief substances reacting with NaOH are the organic acids; for this reason, the titratable acidity represents fairly well the organic acid content of a given juice.

The predominating organic acid in orange juice is citric acid; but other organic acids, namely, tartaric, malic, benzoic, and succinic, have been reported present (2). According to SCURTI and DE PLATO (11), the acidity of the orange is due to citric and malic acids. MENCHIKOVSKY and POPPER (6) have reported that in the grapefruit of Palestine, 98.72 per cent. of the total acid is citric, 0.05 per cent. tartaric, 1.0 per cent. malic, and 0.23 per cent. oxalic. NELSON (7) found that the nonvolatile fruit acids of California lemons were chiefly citric, with a trace of l-malic acid. For Florida orange juice, HARTMANN and HILLIG (5) reported a citric acid concentration of 0.92 per cent. and a malic acid concentration of 0.18 per cent., fresh weight. HALL and WILSON (3) identified formic acid in the volatile fraction of orange juice.

All of these acids, if present in the juice, would have an additive influence on the titratable acidity. It was therefore decided to determine the amounts of citric and malic acids in orange juice and correlate these with the free-acid content as determined by titration with standard NaOH. Undoubtedly, other organic acids are present in the juice in minute amounts, but in the present work it has been assumed that they are negligible in comparison with the concentrations of citric and malic acids. Furthermore, the concentrations of combined and free acids have been determined on various juice samples, and the relation between the combined acid and the inorganic cations in the ash of orange juice has been ascertained.

Materials and methods

For each experiment, aliquot portions of centrifuged juice were taken from the volume of juice reamed from 50 mature Valencia or navel oranges. Total soluble solids were determined with an Abbé refractometer, the refractive indexes being converted to soluble solids by means of a sucrose table. The free-acid content of the juice samples was determined by three different methods: (1) by titration, with phenolphthalein as indicator; (2)

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by potentiometric titration; and, (3) by the lead acetate precipitation method of HARTMANN and HILLIG (4).

In the potentiometric titration, the acidity was calculated from the inflection point of the titration curve. For each sample of juice, the inflection point was determined for the titration curve by plotting the ratio $\frac{\Delta \text{pH}}{\Delta \text{ml.}}$

against the milliliters of NaOH added. All pH values were determined at 23° C., with a Beckman glass-electrode pH meter. In the method of HARTMANN and HILLIG (4), the addition of lead acetate to the juice precipitates the neutral lead salts of the fruit acids, with the formation of an equivalent quantity of acetic acid. The salts of the fruit acids are also precipitated by lead acetate, but this is apparently a replacement reaction, for no acetic acid is liberated in the process. The liberated acetic acid was determined in the dealed filtrate by titrating an aliquot portion with standard NaOH solution. The results were calculated and reported as citric acid.

The actual amount of citric acid and citrates in the juice samples was determined by the method of PUCHER, VICKERY, and LEAVENWORTH (8). The sample was heated with H_2SO_4 to convert the combined citrates to free citric acid, and the citric acid was oxidized to pentabromacetone by KMnO_4 in the presence of KBr . After extraction of the pentabromacetone with petroleum ether, the bromide ion was liberated with Na_2S , and subsequently titrated with standard AgNO_3 . The citric acid in the original sample was calculated from the titration.

The malic acid was precipitated from the juice by a procedure described by HARTMANN and HILLIG (5). The organic acid fraction was precipitated from an alcoholic solution (75 per cent.) with lead acetate, and the precipitate separated from the filtrate by centrifuging. The precipitate was washed with alcohol, suspended in water, and freed of lead by passing H_2S through the solution. The lead sulphide was filtered off and washed with water. The filtrate and washings were combined and diluted to a known volume. The malic acid was determined on aliquot portions of this solution by the method of PUCHER, VICKERY, and WAKEMAN (9). The principle of the method involved the oxidation of malic acid with KMnO_4 , in the presence of KBr , to a bromine compound volatile with steam. This compound reacts with dinitrophenylhydrazine to give a water-insoluble product which is soluble in pyridine. A pyridine solution of this substance, when correctly diluted with water and made alkaline with NaOH, develops a blue color proportional to the amount of malic acid present.

The combined-acid concentration of various juice samples was estimated by determining the alkalinity of the ash from an aliquot of juice. A 50-ml. aliquot of juice was evaporated to dryness on a steam bath, and then charred over a low flame before ashing to a white residue at 450° C. An aliquot of standard HCl was added to the ash, and the excess acid was titrated with NaOH. The alkalinity was calculated from the equivalents of HCl neutralized by the ash. This value represents closely the equivalents of combined acid present in the original sample of juice.

Results

RELATION OF FREE ACIDITY OF ORANGE JUICE TO THE ACID CONSTITUENTS PRECIPITATED WITH LEAD ACETATE

The organic acids and phosphoric acid were precipitated as the neutral lead salts when lead acetate was added to the juice. The free-acid values (as citric) were calculated from the amounts of standard NaOH solution required to neutralize the liberated acetic acid. According to HARTMANN and HILLIG (4), the organic acids and acid salts are precipitated by lead acetate, and free acetic acid is thus liberated. When this liberated acetic acid is determined by titration, the original free-acid content of the juice can be calculated. These values were determined on each sample of juice reported in table I.

The differences in the amounts of acid determined in the juice by direct titration and by the lead acetate method are statistically insignificant. This means that lead acetate precipitates the same acid substances that are neutralized when the juice is titrated directly with standard NaOH to phenolphthalein end point. If the juice contains high acid phosphates, these would be included in the values obtained by titrating the liberated acetic acid in the filtrate from the lead acetate precipitate, whereas, in titrating the juice directly with NaOH, only part of the phosphoric acid would be included. The phosphates occur with the organic acids in fruits as the acid phosphates, and these substances contribute to the titratable acidity. A correction should therefore be made for the acid phosphate in the juice, depending upon the amount present. The second hydrogen of phosphoric acid is slightly undertitrated at the end point of phenolphthalein (pH 8.10). When titrating potassium dihydrogen phosphate electrometrically, BOLLINGER (1) observed that 47.4 per cent. of the total hydrogen ions was neutralized at pH 8.10. When titrating this same material with phenolphthalein, HARTMANN and HILLIG (4) obtained results accounting for 50 per cent. of the total available hydrogen ions. The results of these experiments show that the acid substances determined by the lead acetate method are a true measure of the free acidity of orange juice.

Since the phenolphthalein titration for orange juice is always high, owing to the fact that the indicator gives a color change above the inflection point of the titration curve, it would appear to be difficult to compare the free acidity (as citric acid) obtained by direct titration and by the lead acetate method. However, when the various factors are studied carefully, it is evident that there is considerable basis for comparison. Probably the most important of these factors is the influence of small amounts of acid phosphates in orange-juice samples.

When orange juice is titrated potentiometrically, all free acids and approximately 40 to 45 per cent. of the acid phosphate salts are neutralized at the inflection point of the juice. The difference in acidity between the lead acetate value and the potentiometric value must therefore be due to acid salts, such as phosphates, that are not completely neutralized at the inflection

TABLE I
COMPARISON OF TOTAL FREE ACID, AS DETERMINED BY DIFFERENT METHODS, AND CITRIC ACID CONTENT OF ORANGE JUICE

SAMPLE NO. AND TYPE	SOLUBLE SOLIDS	SPECIFIC GRAVITY	PH	TOTAL FREE ACID (AS CITRIC ACID) DETERMINED BY				CITRIC ACID (PENTABROMACETONE METHOD)	
				PHENOLPHTHALEIN TITRATION		LEAD ACETATE METHOD		POTENTIOMETRIC TITRATION	
				mg./ml.	% *	mg./ml.	% *	mg./ml.	% *
1. Valencia	12.58	1.0508	3.50	11.8	1.12	11.8	1.12	12.0	1.14
2. Valencia	12.58	1.0508	3.55	12.4	1.18	12.5	1.19	13.1	1.25
3. Navel	16.77	1.0687	3.96	8.3	0.78	8.3	0.78	8.9	0.83
4. Navel	16.32	1.0667	3.97	7.7	0.72	7.8	0.73	8.9	0.83
5. Valencia	11.91	1.0480	3.40	11.3	1.08	11.3	1.08	11.8	1.13
6. Valencia	15.44	1.0630	3.41	13.8	1.30	13.9	1.31	14.7	1.38
7. Valencia	12.31	1.0496	3.23	12.3	1.17	12.4	1.18	12.7	1.21
8. Valencia	12.51	1.0505	3.32	12.1	1.15	12.1	1.15	12.0	1.13
9. Valencia	14.32	1.0581	3.45	12.7	1.20	12.7	1.20	13.4	1.27
10. Valencia	13.92	1.0564	3.52	12.8	1.21	12.8	1.21	13.5	1.28
11. Navel	15.72	1.0641	3.99	8.5	0.80	8.4	0.79	9.8	0.92
12. Valencia	13.07	1.0528	3.40	11.4	1.07	11.6	1.09	12.4	1.18
13. Valencia	13.18	1.0533	3.45	11.4	1.08	11.3	1.07	12.7	1.21
14. Valencia	12.51	1.0505	3.80	9.4	0.89	9.5	0.90	9.6	0.91

* Percentage fresh weight.

points on the titration curves but are precipitated by lead acetate. Some of this difference may be due to other factors, such as slight overtitration of the acetic acid; but it is apparent that in a substance such as orange juice, which contains a small amount of phosphate and other acid salts, the electrometric titration does not account for all of the hydrogen ions. HARTMANN and HILLIG (4) arrived at this same conclusion in their work with jams and jellies and other fruit products.

From the data in table I, it can be seen that the values determined by phenolphthalein titration and by the lead acetate method agree very closely, even though the direct titration value is considered higher than the actual acidity. Evidently, the phenolphthalein titrates just high enough above the actual inflection point of the juice to account for the untitrated acid phosphates. In principle, it amounts to one error compensating for another.

A number of experiments were carried out on the precipitate formed by the addition of lead acetate to the juice. This precipitate included the lead salts of the fruit acids, combined acids, and acid salts of the juice. Quantitative data on these precipitated constituents are given elsewhere in this paper. The precipitate was freed of lead by passing H_2S through the material suspended in water. The insoluble PbS was filtered off, leaving the free acids in the filtrate. The citrates and acid salts which previously existed as such in the natural juice were then in the form of free acids and could be titrated in the absence of buffer salts. The titration curve of these constituents gave a curve similar to that of pure citric acid. This was what would be expected, for the titration of free acids in an unbuffered system, such as that of citric acid, gives a steeper curve near the inflection point than in a highly buffered system such as that of orange juice.

THE FREE ACID OF ORANGE JUICE DETERMINED BY PHENOLPHTHALEIN TITRATION AND BY POTENTIOMETRIC TITRATION

The free acid (as citric) per milliliter of juice, calculated from the volume of standard $NaOH$ required for neutralization, with phenolphthalein as indicator, was higher than the amounts determined by potentiometric titration (tables I, II). Since phenolphthalein changes color between pH 8 and 9, slightly more $NaOH$ is required to reach the end point with phenolphthalein than to titrate the corresponding samples potentiometrically to pH 7.80. Immediately after the juice samples had been titrated with $NaOH$, with phenolphthalein as indicator, the pH values were determined and found to range between pH 8.25 and pH 8.45 (table II). It is essential that the sample be sufficiently diluted with distilled water to prevent the natural coloring matter in the juice from interfering with the end point produced with phenolphthalein. If this precaution is not observed, it is very easy to overtitrate the solution to a pH value ranging from 8.8 to 9.2.

The value for the mean pH and the standard deviation for the inflection points on the titration curves of orange juice was 7.80 ± 0.03 . This value agrees with that of ROBERTS (10), who showed that the inflection points on

TABLE II

COMPARISON OF THE PHENOLPHTHALEIN AND POTENTIOMETRIC TITRATION METHODS OF DETERMINING THE FREE ACIDITY (AS CITRIC) OF ORANGE JUICE

SAMPLE NO. AND TYPE	PHENOLPHTHALEIN TITRATION		POTENTIOMETRIC TITRATION		CITRIC ACID CALCULATED*
	CITRIC ACID	PH AT END POINT	CITRIC ACID	PH AT INFLEC- TION POINT	
	<i>mg./ml.</i>		<i>mg./ml.</i>		<i>mg.</i>
1. Navel	8.6	8.28	8.2	7.82	8.5
2. Navel	10.0	8.45	9.4	7.79	9.9
3. Valencia	10.8	8.40	10.4	7.80	10.7
4. Valencia	10.3	8.25	9.9	7.82	10.1
5. Valencia	9.7	8.44	9.2	7.84	9.5
6. Navel	9.1	8.36	8.6	7.79	9.0
7. Valencia	11.7	8.41	11.2	7.80	11.6
8. Valencia	11.1	8.43	10.6	7.77	10.9

* Calculated by taking NaOH value from potentiometric curve at point of pH of phenolphthalein end point.

the titration curves of various fruit juices occurred at approximately pH 7.90.

The free acidity was calculated from the amounts of standard NaOH required to bring each sample of juice to the inflection point. Since more alkali was required to bring the juice to the phenolphthalein end point on

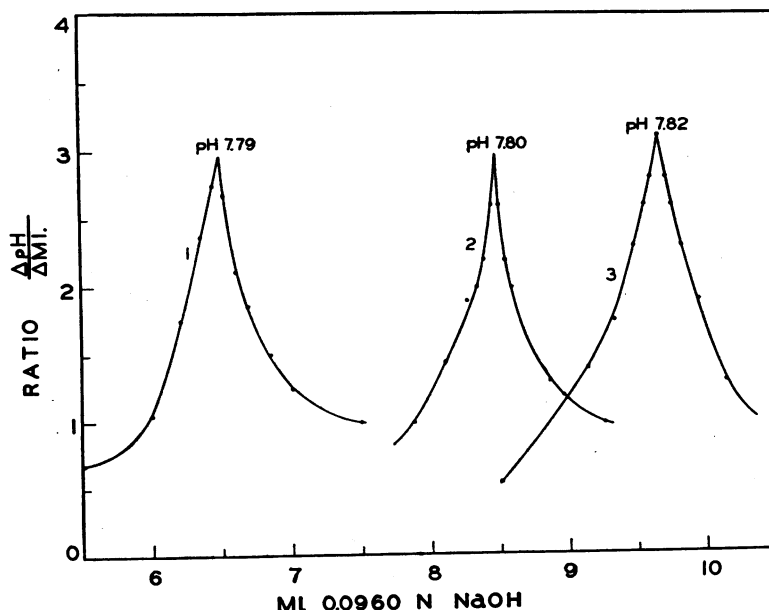


FIG. 1. The inflection points on the titration curves of three typical samples of Valencia-orange juice. The points were determined by plotting the change in ratio $\frac{\Delta \text{pH}}{\Delta \text{ml.}}$ against the milliliters of NaOH added.

the curve (a point between pH 8.25 and 8.45), it is to be expected that the titrations using phenolphthalein as an indicator would yield higher free-acid values (table II). Experimentally, the more accurate values for the amounts of free acid in the juice samples would be given by values calculated from the amounts of NaOH required to bring the samples to the inflection points. These are the values used in determining the combined acids of orange juice.

The inflection points on the titration curves of three typical samples of Valencia-orange juice are shown in figure 1. The values shown on each graph were from the portion of the titration curve near the neutral point

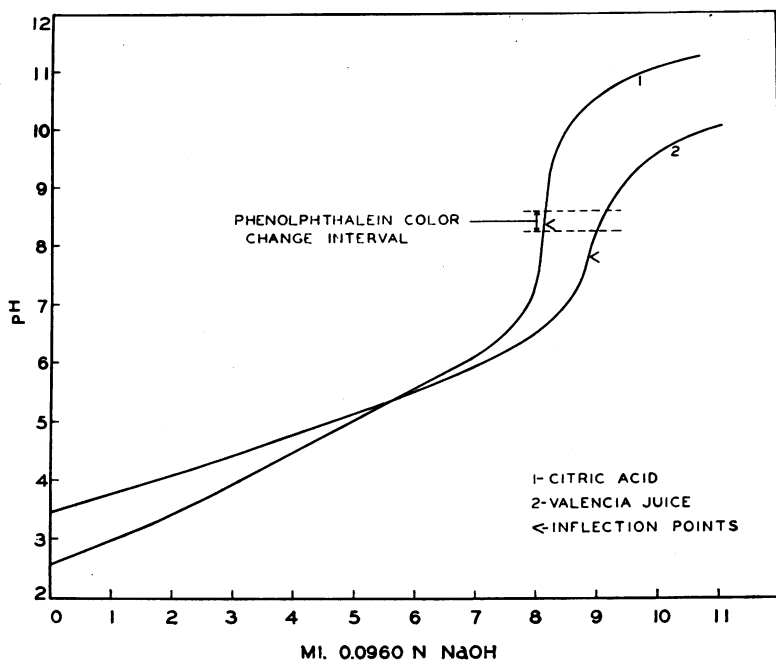


FIG. 2. Comparison of the buffer properties (1) of pure citric acid; and (2) of natural Valencia-orange juice. Note the difference in the inflection points of the two curves and the relation of the inflection points to the phenolphthalein end point.

and were determined by plotting the ratio $\frac{\Delta \text{pH}}{\Delta \text{ml.}}$ against the milliliters of standard NaOH added. This ratio represents the increment of pH for a given addition of NaOH solution. That each of the three samples had different quantities of free acid was shown by the different amounts of NaOH required to bring them to their inflection points (see curves 1, 2, and 3, fig. 1). It is of interest to note the insignificant differences between the pH values of the juice samples at their inflection points.

The acidity of fruits is chiefly due to the polybasic acids which constitute the major portion of the total fruit acids. This is especially true of orange juice, in which total acidity is due mostly to citric acid. As previously noted, the electrometric titration curve of orange juice shows a sharp in-

flection close to pH 7.80. This is the only sharp inflection in the titration curve of orange juice, for the three dissociation exponents (pK_a values) of citric acid are sufficiently close to cause the removal of the multiple inflections on the curve. In general, the titration curves of extracts of most acid fruits show a sharp inflection close to pH 8.0. According to the experiments of BOLLINGER (1), the total acidities of juice and water extracts of fruits, calculated from electrometric end points of pH 8.10, agreed closely with those determined by the phenolphthalein titration.

The titration curve of pure citric acid (fig. 2) is nearly perpendicular to the abscissa in that portion of the curve close to the neutral point. The titration curve of orange juice shows considerable slope within a similar region of the curve. It can be seen that the titration curve of pure citric acid would produce a much larger ratio $\frac{\Delta \text{pH}}{\Delta \text{ml.}}$ at the inflection point than would orange juice. In all the samples studied, the value of the ratios $\frac{\Delta \text{pH}}{\Delta \text{ml.}}$ at the inflection points varied almost 100 per cent. between samples. Unlike the orange-juice titration, the phenolphthalein indicator gives an accurate end point when pure citric acid is titrated with NaOH. This is to be expected because all buffering agents of any importance are absent, and the titration curve is almost perpendicular near the inflection point. Since the titration curve of orange juice is not so steep near the inflection point, it is evident that more NaOH would be required to bring such a highly buffered solution to the phenolphthalein end point.

THE CITRIC ACID CONTENT OF ORANGE JUICE

The amounts of citric acid (milligrams per milliliter) actually determined in the different juice samples are shown in table I. It should be pointed out that the citric acid values, as determined by the method of PUCHER, VICKERY, and LEAVENWORTH (8), include both the free citric acid and that combined in the form of citrates. These values are higher than the quantity of free acid calculated as citric acid from the amount of standard NaOH required to bring the sample to pH 7.80 on the electrometric titration curve; they are also slightly higher than the amounts determined from the titratable acidity. The data reported for the actual amount of citric acid in the juice represent the major portion of the total organic acid content of orange juice.

The degree of accuracy with which the citric acid and the citrate content of orange juice can be determined by the pentabromacetone method is reported in table III. The data given include the determination of citric acid on a solution of known strength, and on aliquot portions of this solution, to which increments of 1 to 5 ml. of sodium citrate solution had been added. The concentration of the sodium citrate solution was prepared so that 1 ml. of the solution was equivalent to 1 mg. of citric acid. Similar determinations were made on samples of orange juice to which increments of sodium

citrate had been added. It can be seen that the amount of citrate added, both to the standard solutions and to the orange-juice samples, could be quantitatively determined by the pentabromacetone method. The values obtained by this method therefore include both the free citric acid and the combined citrates. It follows that the difference between the actual citric acid value and an accurate measure of the free acid represents the citrate content of a given juice sample only when all the free acid in the juice is citric acid.

THE MALIC ACID CONTENT OF ORANGE JUICE

The malic acid content of various orange-juice samples is recorded in table IV. The citric acid values are reported for the same samples; and for comparison, these values were obtained on the pure juice and on the acid

TABLE III

RECOVERY OF CITRIC ACID AND ADDED SODIUM CITRATE FROM DIFFERENT
SAMPLES OF ORANGE JUICE

SAMPLE	MILLIGRAMS CITRIC ACID PER MILLILITER, DETERMINED BY						
	PHENOL- PHTHALEIN TITRATION	LEAD ACE- TATE PRE- CIPITATION	POTENTIO- METRIC TITRATION	PENTABROMACETONE METHOD, ON SAMPLES HAVING GIVEN AMOUNTS OF SODIUM CITRATE ADDED*			
				None	1 ml.	3 ml.	5 ml.
	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>	<i>mg.</i>
Standard citric acid	10.0	10.0	10.0	10.0	11.1	13.1	15.0
Valencia-orange juice	12.7	12.7	12.1	13.4	14.5	16.5	18.4
Valencia-orange juice	12.8	12.8	12.2	13.5	14.4	16.6	18.6
Navel-orange juice	8.5	8.4	7.5	9.8	10.7	12.9	14.9

* Sodium citrate was a concentration equivalent to 1 mg. citric acid per milliliter. The increments of sodium citrate solution were added to 1 ml. of juice.

constituents precipitated with lead acetate. The degree of maturity of the fruit from which the samples were taken can be partly indicated by the soluble-solids content, pH of the juice, and the acidity. The slight variation in the malic acid content of the different samples, in contrast to the large differences occurring in corresponding amounts of citric acid, is noteworthy. For example, the malic acid values for samples 3, 4, and 5 were 1.58, 1.40, and 1.58 mg. per milliliter, respectively, whereas the citric acid values for these samples were 25.51, 9.27, and 12.99 mg. per milliliter. As the amounts of malic acid in the juice samples are fairly constant, the differences occurring in free acidity (potentiometric titration) are due chiefly to differences in their citric acid contents.

Attention should be drawn to the close agreement between the citric acid values determined on pure juice and those determined on substances precipitated with lead acetate. Apparently, lead acetate gave a complete precip-

TABLE IV
COMPARISON OF CITRIC ACID AND MALIC ACID CONTENT OF ORANGE JUICE

SAMPLE NO. AND TYPE	PURE JUICE			ACID CONSTITUENTS PRECIPITATED BY LEAD ACETATE				COMBINED ACID (AS CITRIC)	FREE ACID PERCENTAGE OF TOTAL ACID RADICALS*	
	SOLUBLE SOLIDS	PH	CITRIC ACID DETERMINED BY		CITRIC ACID (PENTABROM- ACETONE METHOD)	MALIC ACID				
			POTENTIOMETRIC TITRATION	PENTABROM- ACETONE METHOD						
	%	mg./ml.	m.e. [†] /ml.	mg./ml.	m.e./ml.	mg./ml.	m.e./ml.	mg./ml.	m.e./ml.	%
1. Navel†	11.23	12.28	0.192	13.83	0.216	13.59	0.015	2.50	0.039	83.1
2. Valencia	15.38	7.98	0.126	9.53	0.149	9.51	0.022	2.88	0.045	73.7
3. Valencia	10.57	24.18	0.378	25.39	0.397	25.51	0.024	2.75	0.043	89.8
4. Valencia	14.78	7.80	0.122	9.42	0.147	9.27	0.021	2.94	0.046	72.7
5. Navel	13.24	11.45	0.179	12.97	0.203	12.99	0.024	3.07	0.048	78.8
6. Navel	12.84	13.03	0.204	14.82	0.231	14.83	0.026	3.39	0.053	79.4
7. Valencia	14.97	6.87	0.107	8.38	0.131	8.28	0.021	2.88	0.045	70.4
8. Navel	12.17	11.17	0.174	12.42	0.194	12.46	0.026	2.94	0.046	79.3
9. Valencia	10.83	23.38	0.365	24.72	0.386	24.60	0.024	2.88	0.045	89.1

* The percentage of free acid is calculated from the ratio $\frac{\text{free acid} \times 100}{\text{total acids}}$, in which free acid indicates milliequivalents of citric acid calculated from the potentiometric titration of the juice. The total acid value is the sum of the milliequivalents of total citric acid (pentabromacetone method) and total malic acid.

† m.e. = milliequivalents.

‡ With the addition of 1 mg. of malic acid per milliliter of juice, malic acid recovery for this sample was 1.99 mg. per ml.

itation of the citric acid radical (free and combined) in the juice. Malic acid determinations are recorded only for the lead acetate precipitate, for in the pure juice there were substances which yielded values on the blank determinations greatly in excess of the actual amount of malic acid in the samples. That lead acetate precipitated all the malic acid was demonstrated by adding known amounts of the acid to juice samples and subsequently determining the malic acid in the lead acetate precipitate of these samples, as shown in table IV, sample 1. There is reason to believe that these malic acid values represent to a fair degree of accuracy (within 5 per cent.) the actual amount of malic acid in the juice samples.

FREE AND COMBINED ORGANIC ACIDS OF ORANGE JUICE

The relative amounts of free acid in the different juice samples are recorded in table IV. The percentage of free acid is calculated from the ratio $\frac{\text{free acid} \times 100}{\text{total acids}}$, in which free acid indicates milliequivalents of citric acid calculated from the potentiometric titration of the juice. The total acid value is the sum of the milliequivalents of total citric acid (determined by the pentabromacetone method) and total malic acid. The concentration of the individual organic acids in the free-acid fraction is unknown; the free acid is therefore calculated in terms of citric acid. The error in this calculation is insignificant, since the malic acid content is low in comparison with the citric acid content, and the equivalent weights of the two acids are nearly equal (citric acid, 64.02; malic acid, 67.02). Total citric acid and total malic acid are calculated as the pure acids, even though part of each exists as the acid salts. When the absolute amounts of these acid salts are given, the values represent the weights of pure citric or malic acid that are combined with the cation, and not the absolute weight of the salt itself.

The relation of pH value to the percentage of free acid in different samples of orange juice is shown graphically in figure 3, curve A. The pH range of this curve shows that the juice samples were taken from orange fruits that ranged from green to overmature. Each point on this curve represents a different juice sample. Curve B shows the percentage of free acid existing at different pH values on a titration curve of pure citric acid (0.2 N). With each increment of NaOH added to the citric acid, the concentration of free acid decreased, while that of the citrate salt increased. The apparent difference between these two curves can be partly explained by the inorganic salts occurring naturally in the juice. The combined effect of these other constituents probably causes a variation in pH away from that of a pure solution of citric acid and its salt.

Within the pH range in which orange fruits are generally accepted as being mature (pH 3.2 to 3.8), the relation (fig. 3, A) is a straight-line function. Both above and below this pH range, the line is more or less curved. The curve on the upper part of the line is probably due to the fact that fruits giving such a high pH value are very near the decomposition point.

In fact, the highest point on that curve represents a sample of fruit in which breaking down of the pulp had already started, and probably is not representative. The reason for the change in slope of curve A at about pH 3.2 has not been experimentally determined. However, if the straight portion of the curve were extended downward with the same slope, it would eventually cross the curve for pure citric acid and citrate.

The pH of even very green fruits never approached that of pure citric acid of the same concentration. With a large number of juice samples there would probably be some variation on either side of the curve. This could be caused by variations in juice constituents, other than the organic acids

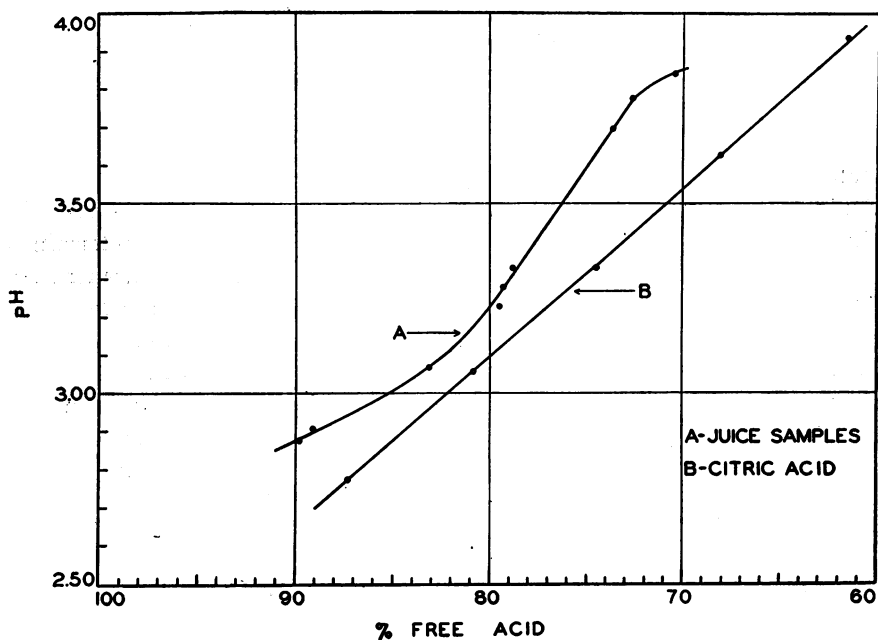


FIG. 3. The relation between pH and the percentage of free acid in orange juice (A), compared with similar data determined on citric acid solution (B). The percentage of free acid is the percentage of the total acid radical that exists as free organic acid. Each dot on curve A represents one of the juice samples listed in table IV, for which the pH, free-acid concentration, and total acid concentration were determined.

and their salts, that affect the pH, and also by the experimental error involved in determining the amounts of free and combined acid in a juice. For example, a small amount of acid phosphate is titrated when the free acidity of a juice is determined potentiometrically. If a correction were made for this phosphate, the free acidity value for the organic acids would be lowered slightly. This would cause curve A to fall closer to curve B. No correction for the acid phosphate was made in this work, because this constituent varies in samples from different districts and even from different trees.

The magnitude of the ratio of combined acid to free acid, at different

pH values of orange juice and of pure citric acid, is shown in figure 4. The initial points on curves B and C designate the values of this ratio at the pH of natural juice before titration. The other points on the curves were obtained by potentiometric titration of the juice sample with standard NaOH. For each increment of alkali, the amount of free acid, in milliequivalents, that was changed to sodium citrate was easily calculated. The combined acid for all points, other than the initial ones, included the combined acid present in the original sample and the sodium citrate formed by the neutralization of part of the free acid. The maximum free-acid concentration

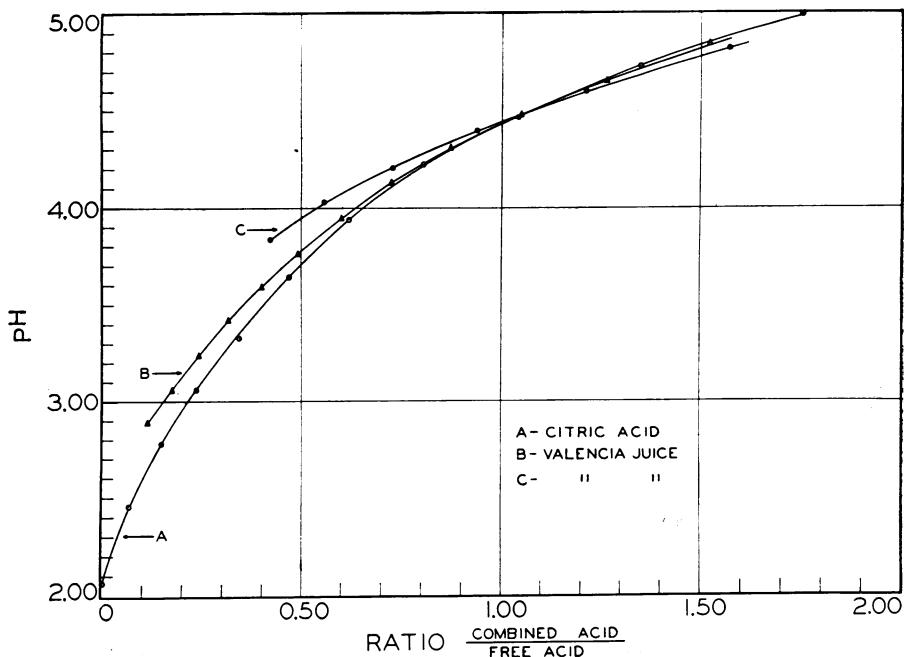


FIG. 4. The relation of pH to the ratio $\frac{\text{combined acid}}{\text{free acid}}$, for two juice samples, compared with a similar curve for pure citric acid solution. The first dot for curves B and C represents the values determined on each juice sample before the addition of any NaOH. For the other points, the term "combined acid" includes the amount occurring in the original sample and the sodium citrate formed by the addition of standard NaOH in titrating the sample potentiometrically.

of the sample is given by the titer value of NaOH required to reach the inflection point. In these studies, the free acid was calculated as milliequivalents of citric acid, and the combined acid as the milliequivalents of citric acid in salt form. As indicated by the initial pH of the juice, samples B and C were from immature and overmature fruit, respectively. Curve B agreed more closely than curve C with the curve for pure citric acid (A). This is undoubtedly due to the fact that the juice from green fruit contained nearly three times as much acid as that from overmature fruit. As the acid content decreased, the acid salts and the other constituents in the

juice had an increasing effect upon the pH, even though their concentration remained nearly constant. It is of interest that the three curves converge at a point where the ratio is almost equal to 1. In other words, when NaOH has been added so that the milliequivalents of free acid and combined acid are equal, the solutions have nearly the same pH. This pH value (4.40 to 4.50) is in close agreement with the dissociation exponent of the second dissociation constant for citric acid ($pK_{a_2} = 4.39$).

If the free- and total-acid values are known, it is a matter of simple calculation to determine, indirectly, the amount of organic acids in the combined form. Experimentally, the problem involves the determination of the individual organic acids and other acid radicals which compose the total acidity (free- and combined-acid radicals). The total organic acid content of the juice was taken as the sum of the milliequivalents of total citric acid

TABLE V

COMBINED-ACID CONTENT OF ORANGE JUICE, DETERMINED FROM ALKALINITY OF ASH

SAMPLE NO. AND TYPE	SOLUBLE SOLIDS	pH	TOTAL FREE ACID (AS CITRIC) DETERMINED BY POTENTIOMETRIC TITRATION		COMBINED ACID (AS CITRIC) CALCULATED FROM CARBONATE CONTENT OF ASH	
			mg./ml.	m.e.* /ml.	mg./ml.	m.e. /ml.
1. Valencia	10.57	2.88	24.18	0.378	2.77	0.043
2. Valencia	14.97	3.84	6.87	0.107	2.87	0.045
3. Valencia	10.83	2.91	23.38	0.365	2.70	0.042
4. Navel	11.31	3.36	10.43	0.163	2.71	0.042
5. Navel	12.78	3.45	9.62	0.150	2.66	0.042
6. Valencia	14.38	3.63	8.44	0.132	2.48	0.039
7. Valencia	12.37	3.03	19.02	0.297	2.92	0.046
8. Valencia	13.07	3.07	16.45	0.257	2.89	0.045

* m.e. = milliequivalents.

and total malic acid determined on each sample. There are undoubtedly minute quantities of other organic acids present, but the concentration of these acids is so small as to be negligible. Therefore, the calculations in table IV were based on the following formula (in milliequivalents):

$$\text{Total acids} = (\text{free citric acid} + \text{free malic acid}) + (\text{combined citric acid} + \text{combined malic acid})$$

With this formula, the amount of combined acid was determined by difference.

The data in table IV show that the amount of combined acid in the different juices was remarkably uniform, the juice from overripe fruit containing no more than the juice from green fruit. Apparently, the free acids are not converted to combined acids, for the absolute amount of free acids actually decreased as the fruit matured. This is further substantiated by calculating the combined acids from the alkalinity of the ash of the juice (table V). The alkalinity of the ash is a measure of the inorganic bases combined with the organic acid radicals, which decompose to carbonate on ashing. The carbonate in the ash is equivalent to a mean of 0.043 milli-

equivalents of acid (2.76 mg. citric acid) in the combined form, per milliliter of juice. The combined acids determined by these two methods (tables IV and V) therefore agreed satisfactorily.

It is apparent that the amount of organic acids that combine with the mineral bases to form salts depends upon the concentration of available cations. The total cation content of the juice is derived from the ash constituents, but not all of these constituents are combined with the organic acids. Some are combined with anions such as the phosphates, sulphates, and a small amount of chlorides. As shown in table I, the citric acid calculated from the titration value, with phenolphthalein as indicator, was significantly lower than the total amount of citric acid and citrates in the juice. Greater differences occurred between the actual amount of citric acid and the amount determined from the electrometric titration curve (at $\text{pH } 7.80 \pm 0.03$) of the various samples. It is obvious, therefore, that the amount of free organic acids available for salt formation in the juice would never be a limiting factor, for the acid concentration at all times would be many times greater than the total cation content.

Summary

The total free acids in the juice of mature Valencia and navel oranges were calculated (1) from the titratable acidity, with phenolphthalein as indicator; (2) from the amount of NaOH required to bring a known volume of orange juice to the equivalence point ($\text{pH } 7.80 \pm 0.03$), when titrating potentiometrically; and (3) from the amount of NaOH required to neutralize the liberated acetic acid formed when the organic acids and acid salts in the juice are precipitated by lead acetate. The values determined by these three methods are compared with the actual amount of citric acid and combined citrates chemically determined in the various samples.

The acid constituents of orange juice, as determined by titrating the juice directly with standard NaOH to phenolphthalein end point, were equivalent to the amounts determined by the lead acetate method. The total free acids determined from the points of inflection on the titration curves were significantly lower than those reported both for the phenolphthalein titration and for the lead acetate method. When juice samples were titrated, with phenolphthalein as indicator, the end points ranged from $\text{pH } 8.25$ to 8.45 . The potentiometric values have been taken to represent more accurately the total acidity.

The concentration of malic acid in different juice samples varied only slightly, as compared with the changes in the citric acid content. With one exception, the samples studied had a malic acid content of from 1.40 to 1.77 mg. per milliliter of juice, while the citric acid varied from 8.38 to 25.39 mg. per milliliter. It is evident that variations in acidity of the orange are due chiefly to changes in the citric acid concentration.

Apparently, there is a definite relation between the free acid-combined acid balance and the pH of the juice. The amount of combined acids in the

juice is remarkably uniform; this indicates that the free-acid concentration is the chief variable. With a decrease in the value of $\frac{\text{total free acid}}{\text{total free and combined acids}}$, a corresponding rise in pH occurs.

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